Amendments to the Specification:

Please replace the table beginning at page 8, line 4 with the following new table:

Table I: Some mutated human hemoglobins
(Int. Hemoglobin Center, 1995)

Abnormal hemoglobin	Normal residues and positions	Replacement
	□ chain (SEQ ID NO: 31)	
I	16 Lys	Glu
$G_{Honolulu}$	30 Glu	Gln
Norfolk	57 Gly	Asp
M_{Boston}	58 His	Tyr
$G_{Philadelphia}$	68 Asn	Lys
$O_{Indonesia}$	116 Glu	Lys
	□ chain (SEQ ID NO: 33)	
C	6 Glu	Lys
S	6 Glu	Val
$G_{San\ José}$	7 Glu	Gly
E	26 Glu	Lys
$M_{Saskatoon}$	63 His	Tyr
Zurich	63 His	Arg
$M_{Milwaukee}$	67 Val	Glu
D_{Punjab}	121 Glu	Gln
Mequon	41 Phe	Tyr
Providence	82 Lys	Asp

Please replace the paragraph beginning at page 12, line 27 with the following new paragraph:

The nucleic acid sequence encoding α- and β- globin and its variants is normally cDNA. Appropriate sequences are illustrated in Figures 2 and 3 (SEQ ID NO: 30 and SEQ ID NO: 32, respectively), any degenerate sequence can also be used as well as the sequences of the variants as defined above.

Please replace the paragraph beginning at page 17, line 10 with the following new paragraph:

The hemin proteins of the invention can be used in numerous pharmaceutical, cosmetic or industrial applications. The invention relates in particular to pharmaceutical compositions comprising one or more hemin protein(s) according to any one of Claims ~~15 to 23~~ 43 to 52, in association with a physiologically acceptable excipient.

Please replace the paragraph beginning at page 17, line 29 with the following new paragraph:

Various aspects of the invention are illustrated in the figures:

- Figure 1: Iron-containing protoporphyrin III (IX),

- Figure 2: cDNA sequence of human α-globin (423 base pairs; SEQ ID NO: 30), and corresponding protein, (SEQ ID NO: 31),

- Figure 3: cDNA sequence of human β-globin (438 base pairs; SEQ ID NO: 32), and corresponding protein, (SEQ ID NO: 33)

Please replace the paragraph beginning at page 23, line 7 with the following new paragraph:

The co-expression binary plasmid is derived from pBIOC21. It contains two expression cassettes each consisting of a pd35S promoter and a 35S polyA terminator but differ in the

polylinker separating the promoter from the terminator. One of the expression cassettes is that of pBIOC21 already described in paragraph I.a. The other expression cassette was obtained by replacing the HindIII-BamHI-SmaI-EcoRI polylinker of pJIT163D (described in paragraph I.a.) by a HindIII-EcoRI adaptor carrying the PacI, AscI, MluI and HpaI restriction sites. This adaptor was obtained by renaturation of the 2 oligodeoxynucleotides WD11 (5' AGC TGA TTA ATT AAG GCG CGC CAC GCG TTA AC 3'; SEQ ID NO: 1) and WD12 (5' AAT TGT TAA CGC GTG GCG CGC CTT AAT TAA TC 3'; SEQ ID NO: 2) which are complementary for their 28 terminal 3' nucleotides. One hundred μM of each of these two oligodeoxynucleotides were previously phosphorylated by the action of 10 U of T4 polynucleotide kinase enzyme (New England Biolabs) in a total reaction volume of 10 μl of 10x T4 polynucleotide kinase buffer (New England Biolabs) and 3μl of ATP (95 mM). The two reaction mixtures were incubated at 37°C for 1 hour, and then at 65°C for 20 min. They were then combined and their volume of phenol;chloroform:isoamyl alcohol (25:24:1) and 1 volume of chloroform:isoamyl alcohol (24:1), 50 μl of 3M sodium acetate pH 6.0 were added. The reaction mixture was incubated at 80°C for 10 min and then cooled slowly to room temperature. The DNA was then precipitated in the presence of 2.5 volumes of absolute ethanol at -80°C for 30 min, centrifuged at 14000 g at 4°C for 1 hour, washed with 70% ethanol, centrifuged at 14000 g at 4°C for 10 min, dried, taken up in 10 μl of H2O. The HindIII-EcoRI DNA fragment was then cloned at the HindIII-EcoRI sites of the plasmid DNA pJIT163D previously dephosphorylated with the enzyme calf intestinal alkaline phosphatase (New England Biolabs) according to the manufacturer's recommendations. The ligation reaction was carried out in a reaction volume of 20 μl in the presence of 1 U of T4 DNA ligase (Gibco-BRL) for a total DNA concentration of 8.5 nM with a vector/insert molar ratio of 1 and of 4 μl of 5x T4 DNA ligase buffer (Gibco-BRL) at 25°C for 16 hours. The E. coli DH5□ bacteria previously made competent were transformed (Hanahan, 1985). The plasmid DNA of the clones obtained, selected on 100 μg/ml ampicillin, was extracted according to the alkaline lysis method (Stephen et al., 1990) and analyzed by enzymatic digestion. The resulting clone was called pBIOC42. Its validity was verified by sequencing with the aid of the "~~Sequenase~~ SEQUENASE™ Version 2.0 DNA Sequencing" kit marketed by United States

Biochemical (USB) according to the dideoxynucleotides method (Sanger et al., 1977). The reaction conditions follow the manufacturer's recommendations except for the denaturation and hybridization. The reaction medium containing the plasmid DNA (0.5 to 1 pmol), the oligonucleotide primer (2pmol), 10% DMSO and the 1x reaction buffer (USB), is incubated at 100°C for 10 min, then suddenly cooled to -80°C in dry ice.

Please replace the paragraph beginning at page 24, line 17 with the following new paragraph:

From pBIOC42, the DNA fragment encoding the expression cassette consisting of the pd35S promoter and of the 35S polyA terminator was isolated by double digestion with SacI and XhoI. It was purified by electrophoresis on a 0.75% agarose gel, and then subjected to the action of the "~~Geneclean~~ GENECLEAN™ II" kit marketed by BIO101 according to the manufacturer's recommendation. Next, this DNA fragment was inserted at the SacI and XhoI sites of the plasmid pBCSK+ marketed by Stratagene and previously dephosphorylated with the enzyme calf intestinal alkaline phosphatase (New England Biolabs) according to the manufacturer's recommendations. The ligation was carried out in a reaction volume of 20 µl in the presence of 1 U of T4 DNA ligase (Gibco-BRL) for a total DNA concentration of 8.5 nM with a vector/insert molar ratio of 1 and of 4 µl of 5x T4 DNA ligase buffer (Gibco-BRL) at 25°C for 16 hours. The E. coli DH5α bacteria previously made competent were transformed (Hanahan, 1985). The plasmid DNA of the clones obtained, selected on 30 µg/ml of chloramphenicol, was extracted according to the alkaline lysis method (Stephen et al., 1990) and analyzed by enzymatic digestion with restriction enzymes. The resulting plasmid was called pBIOC75.

Please replace the paragraph beginning at page 25, line 3 with the following new paragraph:

From pBIOC75, the DNA fragment carrying the expression cassette consisting of the pd35S promoter and the 35S polyA terminator was isolated by digestion with KpnI. It was purified by electrophoresis on a 0.75% agarose gel, and then subjected to the action of the "

~~Geneclean~~ GENECLEAN™ II" kit marketed by BIO101 according to the manufacturer's recommendations. Next, this DNA fragment was ligated to the plasmid DNA of pBIOC21 digested with KpnI and dephosphorylated with the enzyme calf intestinal alkaline phosphatase (New England Biolabs) according to the manufacturer's recommendations. The ligation was carried out in a reaction volume of 20 µl in the presence of 1 U of T4 DNA ligase (Gibco-BRL) for a total DNA concentration of 8.5 nM with a vector/insert molar ratio of 1 and of 4 µl of 5x T4 DNA ligase buffer (Gibco-BRL) at 25°C for 16 hours. The E. coli DH5α bacteria previously made competent were transformed (Hanahan, 1985). The plasmid DNA of the clones obtained, selected on 12 µg/ml of tetracycline, was extracted according to the alkaline lysis method (Stephen et al., 1990) and analyzed by enzymatic digestion with restriction enzymes. The resulting plasmid was called pBIOC43.

Please replace the paragraph beginning at page 26, line 10 with the following new paragraph:

The first stage consisted in the amplification of the first 95 codons of the mature □ globin chain on the plasmid alpha1pJW101 with the aid of the 2 oligodeoxynucleotides, WD13 (5' tacaagcttaaca ATG GTG CTG TCT CCg GCC GAC 3'; SEQ ID NO: 3) and AD27 (5' CGG GTC CAC CCG GAG CTT GTG 3'; SEQ ID NO: 4). The WD13 primer provides the HindIII restriction site, the sequence aaca favoring the initiation of translation (Joshi, 1987) and preceding the initiator ATG codon followed by the first 6 condons of the mature □ globin chain of which the fourth (CCT) is substituted for CCg (silent mutation) in order to create the EagI restriction site. The AD27 primer allows the suppression of the HindIII restriction site by substitution of nucleotide T for C (position 276 of the coding sequence). The PCR amplification was carried out in 100 µl of reaction medium containing 10 µl of 10x Taq DNA polymerase buffer (100 mM Tris-HCl pH 8.4, 500 mM KCl and 20 mM $MgCl_2$), 16 µl of the dNTP mixture (1.25 mM dATP, 1.25 mM dCTP, 1.25 mM dGTP and 1.25 mM dTTP), 10 µl of each of the primers described above at 10 µl, 10 µl of template DNA (alpha1pJW101) at 1 ng/µl and 0.5 µl of Taq DNA polymerase at 5 U/µl (Perkin Elmer). Thirty cycles each comprising 30 sec of

denaturation at 97°C, 1 min hybridization at 55°C and 2 min extension at 72°C were carried out in the Appligène "~~Crocodile~~ CROCODILE™ II" apparatus. The amplified DNA fragments were then purified by electrophoresis on a 1.8% agarose gel and by the action of the "~~Geneclean~~ GENECLEAN™ II" kit marketed by BIO101 according to the manufacturer's recommendations. The purified amplified DNA fragments are taken up in 20 μl.

Please replace the paragraph beginning at page 27, line 3 with the following new paragraph:

The second stage consisted in the amplification of the last 54 codons of the mature □ globin chain on the plasmid alpha1pJW101 with the aid of the 2 oligodeoxynucleotides, AD26 (5' CAC AAG CTC CGG GTG GAC CCG 3'; SEQ ID NO: 5) and WD14 (5' gcgaattc TCA ACG GTA TTT GGA GGT CAG CAC 3'; SEQ ID NO: 6). The WD14 primer provides the EcoRI restriction site situated just after the stop codon. The AD26 primer allows the suppression of the HindIII restriction site by substitution of nucleotide T for C (position 276 of the coding sequence). The PCR amplification was carried out as described in the first stage. The treatment of the amplified DNA fragments was carried out as described in the first stage.

Please replace the paragraph beginning at page 28, line 14 with the following new paragraph:

The cDNA encoding the cytoplasmic targeting β globin chain was obtained by the PCR amplification of the 146 codons constituting the mature β globin chain on the phage M13mp10 cIIFX beta with the aid of the 2 oligodeoxynucleotides WD15 (5' gtcattaattaaca ATG GTG CAC CTG ACT CCT GAG GAG AAG TCg GCC GTT AC 3'; SEQ ID NO: 7) and WD16 (5' aatgagctcgttaacgcgt TTA GTG ATA CTT GTG GGC CAG GGC 3'; SEQ ID NO: 8). The WD15 primer provides the PacI restriction site, the aaca sequence favoring the initiation of translation (Joshi, 1987) and the initiator ATG codon followed by the first 12 codons of the mature β globin chain of which the ninth (TCT) is substituted for TCg (silent mutation) in order to create the EagI restriction site. The WD16 primer provides the MluI, HpaI and SacI

restriction sites placed after the stop codon. The PCR amplification and the treatment of the amplified DNA fragments were carried out as described in the third stage of section II.a. Next, 25 μl of these DNA fragments were doubly digested with PacI and SacI, purified by electrophoresis on a 1.8% agarose gel and by the action of the "~~Geneclean~~ GENECLEAN™ II" kit (BIO101) and cloned at the PacI and SacI sites of the plasmid pNEB193 marketed by New England Biolabs, previously dephosphorylated by the enzyme calf intestinal alkaline phosphatase (New England Biolabs) according to the manufacturer's recommendations. The ligation and the transformation were carried out as described in section I.b. The plasmid DNA of the clones obtained, selected on 100 μg/ml ampicillin, was extracted according to the alkaline lysis method (Stephen et al., 1990) and analyzed by enzymatic digestion. The resulting clone was called pBIOC45. The nucleotide sequence of the cDNA encoding the recombinant β globin chain was verified by sequencing as described in section I.b.

Please replace the paragraph beginning at page 29, line 12 with the following new paragraph:

Starting with pBIOC44, the HindIII-EcoRI fragment carrying the cDNA encoding the cytoplasmic targeting α globin chain was isolated by double enzymatic digestion with HindIII and EcoRI, purified by electrophoresis on a 1.8% agarose gel and by the action of the "~~Geneclean~~ GENECLEAN™ II" kit (BIO101). Next, this DNA fragment was ligated with the plasmid DNA of pBIOC21 doubly digested with HindIII and EcoRI, and dephosphorylated with the enzyme calf intestinal alkaline phosphatase (New England Biolabs) according to the manufacturer's recommendations. The ligation and the transformation were carried out as described in section I.b. The plasmid DNA of the clones obtained, selected on 10 μg/ml tetracycline, was extracted according to the alkaline lysis method (Stephen et al., 1990) and analyzed by enzymatic digestion. The resulting clone was called pBIOC46. The nucleotide sequence of the cDNA encoding the recombinant α globin chain was verified by sequencing as described in section I.b. The plasmid DNA of the binary vector pBIOC46 was introduced by direct transformation into the Agrobacterium tumefaciens LBA4404 strain according to the

method of Holsters et al. (1978). The validity of the clone selected was verified by enzymatic digestion of the plasmid DNA introduced.

Please replace the paragraph beginning at page 30, line 4 with the following new paragraph:

Starting with pBIOC45, the HindIII-EcoRI fragment carrying the cDNA encoding the cytoplasmic targeting β globin chain was isolated by double enzymatic digestion with HindIII (total digestion) and EcoRI (partial digestion), purified by electrophoresis on a 1.8% agarose gel and by the action of the "~~Geneclean~~ GENECLEAN™" kit (BIO101). Next, this DNA fragment was ligated with the plasmid DNA of pBIOC21 doubly digested with HindIII and EcoRI and dephosphorylated with the enzyme calf intestinal alkaline phosphatase (New England Biolabs) according to the manufacturer's recommendations. The ligation and the transformation were carried out as described in section I.b.

Please replace the paragraph beginning at page 31, line 25 with the following new paragraph:

To obtain mitochondrial targeting, the sequence encoding the transit peptide of the Nicotiana plumbaginifolia mitochondrial ATPase-Fi □ subunit precursor (ATG GCT TCT CGG AGG CTT CTC GCC TCT CTC CTC CGT CAA TCG GCT CAA CGT GGC GGC GGT CTA ATT TCC CGA TCG TTA GGA AAC TCC ATC CCT AAA TCC GCT TCA CGC GCC TCT TCA CGC GCA TCC CCT AAG GGA TTC CTC TTA AAC CGC GCC GTA CAG TAC; SEQ ID NO: 9) is fused with the first codon of the sequence encoding, on the one hand, the mature α globin chain (deletion of the initiator ATG) and, on the other hand, the mature β globin chain (deletion of the initiator ATG) while maintaining the open reading frames.

Please replace the paragraph beginning at page 32, line 18 with the following new paragraph:

The sequence encoding the transit peptide of the mitochondrial ATPase-F1 □ subunit precursor was amplified by PCR on the plasmid pTZ-catp2-1 with the aid of the 2 oligodeoxynucleotides, WD17 (5' cgcaagcttaaca ATG GCT TCT CGG AGG CTT CTC 3'; SEQ ID NO: 10) and WD18 (5' tag aat tC GGC cGG AGA CAG CAC GTA CTG TAC GGC GCG GTT TAA G 3'; SEQ ID NO: 11). The WD17 primer provides the HindIII restriction site, the aaca sequence promoting the initiation of translation (Joshi, 1987) and the first 7 codons of the transit peptide (including the initiator ATG). The WD18 primer provides the EcoRI restriction site, the first 5 codons of the sequence encoding the mature α globin chain (an EagI restriction site is created by silent mutation in the fourth codon (CCT modified to CCG) and the last 7 codons of the sequence of the transit peptide. PCR amplification and the treatment of the amplified DNA fragments were carried out as described in the third step of chapter II.a. Next, these DNA fragments were doubly digested with HindIII and EagI, purified by electrophoresis on a 1.8% agarose gel and by the action of the "~~Geneclean~~ GENECLEAN™ II" kit (BIO101) and cloned at the HindIII and EagI sites of the plasmid pBIOC44 described in section II.a., previously purified by electrophoresis on a 0.75% agarose gel and using the "~~Geneclean~~ GENECLEAN™ II" kit. The plasmid pBIOC44 was dephosphorylated by the enzyme calf intestinal alkaline phosphatase (New England Biolabs) according to the manufacturer's recommendations. The ligation and the transformation were carried out as described in section I.b. The plasmid DNA of the clones obtained, selected on 100 μg/ml ampicillin, was extracted according to the alkaline lysis method (Stephen et al., 1990) and analyzed by enzymatic digestion. The resulting clone was called pBIOC50. The nucleotide sequence of this chimeric gene resulting from the translational fusion between the sequence encoding the transit peptide and the cDNA encoding the mature α globin chain was verified by sequencing as described in section I.b. The sequencing revealed two silent mutations situated at the tenth nucleotide (C modified to A) and at the one hundred forty first (C modified to G) of the coding sequence for the transit peptide.

Please replace the paragraph beginning at page 33, line 23 with the following new paragraph:

The sequence encoding the transit peptide of the mitochondrial ATPase-F1 □ subunit precursor was amplified by PCR on the plasmid pTZ-catp2-1 with the aid of the 2 oligodeoxynucleotides, WD19 (5' gtcattaattaaca ATG GCT TCT CGG AGG CTT CTC GCC TCT C 3'; SEQ ID NO: 12) and WD20 (5'aatgagct C GGC cGA CTT CTC CTC AGG AGT CAG GTG CAC GTA CTG TAC GGC GCG GTT TAA G 3'; SEQ ID NO: 13). The WD19 primer provides the PacI restriction site, the aaca sequence promoting the initiation of translation (Joshi, 1987) and preceding the first 9 codons of the transit peptide (including the initiator ATG). The WD20 primer provides the SacI restriction site, the first 10 codons of the sequence encoding the mature β globin chain (an EagI restriction site is created by silent mutation in the ninth condon (TCT modified to TCg)) and the last 7 codons of the sequence of the transit peptide. The PCR amplification and the treatment of the amplified DNA fragments were carried out as described in the third stage of section II.a. Next, these DNA fragments were doubly digested with PacI and EagI, purified by electrophoresis on a 1.8% agarose gel and by the action of the "~~Geneclean~~ GENECLEAN™ II" kit (BIO101) and cloned at the PacI and EagI sites of the plasmid pBIOC45 described in section II.b., previously purified by electrophoresis on a 0.75% agarose gel and using the "~~Geneclean~~ GENECLEAN™ II" kit. The plasmid pBIOC45 was dephosphorylated by the enzyme calf intestinal alkaline phosphatase (New England Biolabs) according to the manufacturer's recommendations. The ligation and the transformation were carried out as described in section I.b. The plasmid DNA of the clones obtained, selected on 100 μg/ml ampicillin, was extracted according to the alkaline lysis method (Stephen et al., 1990) and analyzed by enzymatic digestion. The resulting clone was called pBIOC51. The nucleotide sequence of this chimeric gene resulting from the translational fusion between the sequence encoding the transit peptide and the cDNA encoding the mature β globin chain was verified by sequencing as described in section I.b.

Please replace the paragraph beginning at page 35, line 19 with the following new paragraph:

To obtain chloroplastic targeting, the sequence encoding the transit peptide of the precursor of the small subunit of ribulose 1,5-diphosphate carboxylase of Pisum sativum L. (ATG GCT TCT ATG ATA TCC TCT TCA GCT GTG ACT ACA GTC AGC CGT GCT TCT ACG GTG CAA TCG GCC GCG GTG GCT CCA TTC GGC GGC CTC AAA TCC ATG ACT GGA TTC CCA GTT AAG AAG GTC AAC ACT GAC ATT ACT TCC ATT ACA AGC AAT GGT GGA AGA GTA AAG TGC; SEQ ID NO: 14) is fused with the first codon of the sequence encoding, on the one hand, the mature α globin chain (deletion of the initiator ATG) and, on the other hand, the mature β globin chain (deletion of the initiator ATG) while maintaining the open reading frames.

Please replace the paragraph beginning at page 36, line 12 with the following new paragraph:

The sequence of the transit peptide of the precursor of the small subunit of ribulose 1,5-diphosphate carboxylase was amplified by PCR on the plasmid pJIT117 with the aid of the 2 oligodeoxynucleotides, WD21 (5' cgcaagcttaaca ATG GCT TCT ATG ATA TCC TCT TCA GC 3'; SEQ ID NO: 15) and WD22 (5' tag aat tC GGC cGG AGA CAG CAC GCA CTT TAC TCT TCC ACC ATT GC 3'; SEQ ID NO: 16). The WD21 primer provides the HindIII restriction site, the aaca sequence promoting the initiation of translation (Joshi, 1987) and the first 8 codons of the transit peptide (including the initiator ATG). The WD22 primer provides the EcoRI restriction site, the first 5 codons of the sequence encoding the mature α globin chain (an EagI restriction site is created by silent mutation in the fourth codon (CCT modified to CCg)) and the last 7 codons of the sequence of the transit peptide. The PCR amplification and the treatment of the amplified DNA fragments were carried out as described in the third stage of section II.a.

Please replace the paragraph beginning at page 37, line 4 with the following new paragraph:

From the plasmid pBIOC54, the HindIII-EagI fragment, carrying the sequence encoding the transit peptide of the precursor of the small subunit of ribulose 1,5-diphosphate carboxylase and the first 4 codons of the mature α globin chain was isolated by double digestion, HindIII (total digestion) and EagI (partial digestion). This HindIII-EagI fragment, purified by electrophoresis on a 1.8% agarose gel and by the action of the "~~Geneclean~~ GENECLEAN™ II" kit (BIO101) was cloned at the HindIII and EagI sites of the dephosphorylated plasmid pBIOC44 as described in section II.a. The ligation and the transformation were carried out as described in section I.b. The plasmid DNA of the clones obtained, selected on 100 μg/ml ampicillin, was extracted according to the alkaline lysis method (Stephen et al., 1990) and analyzed by enzymatic digestion. The resulting clone was called pBIOC55. The nucleotide sequence of this chimeric gene resulting from the translational fusion between the sequence encoding the transit peptide and the cDNA encoding the mature α globin chain was verified by sequencing as described in section I.b.

Please replace the paragraph beginning at page 37, line 26 with the following new paragraph:

The sequence encoding transit peptide of the ribulose 1,5-diphosphate carboxylase small subunit precursor was amplified by PCR on the plasmid pJIT117 with the aid of the 2 oligodeoxynucleotides, WD23 (5' gtcattaattaaca ATG GCT TCT ATG ATA TCC TCT TCA GCT GTG 3'; SEQ ID NO: 17) and WD24 (5' aatgagct C GGC cGA CTT CTC CTC AGG AGT CAG GTG CAC GCA CTT TAC TCT TCC ACC 3'; SEQ ID NO: 18). The WD23 primer provides the PacI restriction site, the aaca sequence promoting the initiation of translation (Joshi, 1987) and preceding the first 10 codons of the transit peptide (including the initiator ATG). The WD24 primer provides the SacI restriction site, the first 10 codons of the sequence encoding the mature β globin chain (an EagI restriction site is created by silent mutation in the ninth codon (TCT modified to TCg)) and the last 6 codons of the sequence of the transit peptide. The PCR amplification and the treatment of the amplified DNA fragments were carried out as described in the third stage of section II.a. Next, these DNA fragments were doubly digested with PacI and

SacI, purified by electrophoresis on a 1.8% agarose gel and by the action of the "~~Geneclean~~ GENECLEAN™ II" kit (BIO101) and cloned at the PacI and SacI sites of the plasmid pNEB193 marketed by New England Biolabs. The plasmid pNEB193 was dephosphorylated as described in II.a. The ligation and the transformation were carried out as described in section I.b. The plasmid DNA of the clones obtained, selected on 100 μg/ml ampicillin, was extracted according to the alkaline lysis method (Stephen et al., 1990) and analyzed by enzymatic digestion. The resulting clone was called pBIOC56. The nucleotide sequence of this chimeric gene resulting from the translational fusion between the sequence encoding the transit peptide and the cDNA encoding the mature β globin chain was verified by sequencing as described in section I.b.

Please replace the paragraph beginning at page 38, line 21 with the following new paragraph:

From the plasmid pBIOC56, the PacI-EagI fragment, carrying the sequence of the transit peptide of the ribulose 1,5-diphosphate carboxylase small subunit precursor and the first 9 codons of the sequence encoding the mature β globin chain, was isolated by double digestion, PacI (total digestion) and EagI (partial digestion). This PacI-EagI fragment, purified by electrophoresis on a 1.8% agarose gel and by the action of the "~~Geneclean~~ GENECLEAN™ II" kit (BIO101), was cloned at the PacI and EagI sites of the dephosphorylated plasmid pBIOC45 as described in section II.a. The ligation and the transformation were carried out as described in section I.b. The plasmid DNA of the clones obtained, selected on 100 μg/ml ampicillin, was extracted according to the alkaline lysis method (Stephen et al., 1990), and analyzed by enzymatic digestion. The resulting clone was called pBIOC57. The nucleotide sequence of this chimeric gene resulting from the translational fusion between the sequence encoding the transit peptide and the cDNA encoding the mature β globin chain was verified by sequencing as described in section I.b.

Please replace the paragraph beginning at page 40, line 5 with the following new paragraph:

To obtain secretion, the sequence encoding the signal peptide (SP) of sporamine A of the tuberized roots of sweet potato (Murakami et al., 1986; Matsuoka and Nakamura, 1991) (ATG AAA GCC TTC ACA CTC GCT CTC TTC TTA GCT CTT TCC CTC TAT CTC CTG CCC AAT CCA GCC CAT TCC; SEQ ID NO: 19), is fused with the first codon of the sequence encoding, on the one hand, the mature α globin chain (deletion of the initiator ATG) and, on the other hand, the mature β globin chain (deletion of the initiator ATG) while maintaining the open reading frames. This signal peptide of 23 amino acids was isolated from the plasmid pMAT103 (Matuoka and Nakamura, 1991) and used during the carrying out of the constructions.

Please replace the paragraph beginning at page 40, line 20 with the following new paragraph:

The sequence encoding the signal peptide (SP) of the sporamine A of the tuberized roots of sweet potato was amplified by PCR on the plasmid pMAT103 with the aid of 2 oligodeoxynucleotides, WD25 (5' cgcaagcttaaca ATG AAA GCC TTC ACA CTC GC 3'; SEQ ID NO: 20) and WD26 (5' tagaattC GGC cGG AGA CAG CAC GGA ATG GGC TGG ATT GGG CAG G 3'; SEQ ID NO: 21). The WD25 primer provides the HindIII restriction site, the aaca sequence promoting the initiation of translation (Joshi, 1987) and the first 6 codons of the signal peptide (including the initiator ATG). The WD26 primer provides the EcoRI restriction site, the first 5 codons of the sequence encoding the mature α globin chain (an EagI restriction site is created by silent mutation in the fourth codon (CCT modified to CCg)) and the last 7 codons of the sequence of the signal peptide. The PCR amplification and the treatment of the amplified DNA fragments were carried out as described in the third stage of section II.a. Next, these DNA fragments were doubly digested with HindIII and EagI, purified by electrophoresis on a 1.8% agarose gel and by the action of the "~~Geneclean~~ GENECLEAN™ II" kit (BIO101) and cloned at the HindIII and EagI sites of the dephosphorylated plasmid pBIOC44 described in section II.a. The ligation and the transformation were carried out as described in section I.b.

The plasmid DNA of the clones obtained, selected on 100 μg/ml ampicillin, was extracted according to the alkaline lysis method (Stephen et al., 1990) and analyzed by enzymatic digestion. The resulting clone was called pBIOC60. The nucleotide sequence of this chimeric gene resulting from the translational fusion between the sequence encoding the signal peptide and the cDNA encoding the mature α globin chain was verified by sequencing as described in section I.b.

Please replace the paragraph beginning at page 41, line 21 with the following new paragraph:

The sequence encoding the signal peptide (SP) of the sporamine A of the tuberized roots of sweet potato was amplified by PCR on the plasmid pMAT103 with the aid of the 2 oligodeoxynucleotides, WD27 (5' gtcattaattaaca ATG AAA GCC TTC ACA CTC GC 3'; SEQ ID NO: 22) and WD28 (5' aatgagct C GGC cGA CTT CTC CTC AGG AGT CAG GTG CAC GGA ATG GGC TGG ATT GGG CAG G 3'; SEQ ID NO: 23). The WD27 primer provides the PacI restriction site, the aaca sequence promoting the initiation of translation (Joshi, 1987) and the first 6 codons of the signal peptide (including the initiator ATG). The WD28 primer provides the SacI restriction site, the first 10 codons of the sequence encoding the mature β globin chain (an EagI site is created by silent mutation in the ninth codon (TCT modified to TCg)) and the last 7 codons of the sequence of the signal peptide. The PCR amplification and the treatment of the amplified DNA fragments were carried out as described in the third stage of section II.a. Next, these DNA fragments were doubly digested with PacI and EagI, purified by electrophoresis on a 1.8% agarose gel and by the action of the "~~Geneclean~~ GENECLEAN™ II" kit (BIO101) and cloned at the PacI and EagI sites of the dephosphorylated plasmid pBIOC45 described in section II.b. The ligation and the transformation were carried out as described in section I.b. The plasmid DNA of the clones obtained, selected on 100 μg/ml ampicillin, was extracted according to the alkaline lysis method (Stephen et al., 1990) and analyzed by enzymatic digestion. The resulting clone was called pBIOC61. The nucleotide sequence of this chimeric gene resulting from the translational fusion between the sequence encoding the signal

peptide and the cDNA encoding the mature β globin chain was verified by sequencing as described in section I.b.

Please replace the paragraph beginning at page 43, line 22 with the following new paragraph:

To obtain retention in the endoplasmic reticulum, the sequence encoding the KDEL signal (5' aaa gat gag cta 3'; SEQ ID NO: 24) was placed before the first stop codon (TGA) of the mature α globin chain while maintaining the open reading frame.

Please replace the paragraph beginning at page 43, line 25 with the following new paragraph:

The plasmid containing the cDNA encoding the α globin chain which contains the sequence encoding the KDEL signal placed before its first stop codon was obtained by following the same steps as for the manufacture of the plasmid pBIOC44 described in II.a. except that the WD29 primer (5' gcgaattc TCA tag ctc atc ttt ACG GTA TTT GGA GGT CAG CAC 3'; SEQ ID NO: 25) replaces the WD14 primer. The WD29 primer provides the EcoRI restriction site and the KDEL sequence situated respectively after and before the stop codon.

Please replace the paragraph beginning at page 44, line 19 with the following new paragraph:

To obtain retention in the endoplasmic reticulum, the sequence encoding the KDEL signal (5' aaa gat gag cta 3'; SEQ ID NO: 24) was placed before the first stop codon (TAA) of the mature β globin chain while maintaining the open reading frame.

Please replace the paragraph beginning at page 44, line 22 with the following new paragraph:

The plasmid containing the cDNA encoding the β globin chain which contains the sequence encoding the KDEL signal before its first stop codon was obtained by following the

same steps as for the manufacture of the plasmid pBIOC45 described in II.b., except that the WD30 primer (5'aatgagctcgttaacgcgt TTA tag ctc atc ttt GTG ATA CTT GTG GGC CAG GGC 3'; SEQ ID NO: 26) replaces the WD16 primer. The WD30 primer provides the MluI, HpaI and SacI restriction sites and the KDEL sequence placed respectively after and before the stop codon.

Please replace the paragraph beginning at page 46, line 13 with the following new paragraph:

To allow vacuolar targeting, the sequence encoding the prepropeptide (PPS) of sporamine A of the tuberized roots of sweet potato (Murakami et al., 1986; Matsuoka and Nakamura, 1991), which corresponds to the signal peptide followed by the N-terminal sequence for vacuolar targeting (ATG AAA GCC TTC ACA CTC GCT CTC TTC TTA GCT CTT TCC CTC TAT CTC CTG CCC AAT CCA GCC CAT TCC AGG TTC AAT CCC ATC CGC CTC CCC ACC ACA CAC GAA CCC GCC; SEQ ID NO: 27), is fused with the first codon of the sequence encoding, on the one hand, the mature α globin chain (deletion of the initiator ATG) and, on the other hand, the mature β globin chain (deletion of the initiator ATG) while maintaining the open reading frames. This prepropeptide of 37 amino acids was isolated from the plasmid pMAT103 (Matuoka and Nakamura, 1991) and used during the carrying out of the constructions.

Please replace the paragraph beginning at page 47, line 3 with the following new paragraph:

The sequence encoding the N-terminal prepropeptide (PPS) of the sporamine A of the tuberized roots of sweet potato was amplified by PCR on the plasmid pMAT103 with the aid of the 2 oligodeoxynucleotides, WD25 (5' cgcaagcttaaca ATG AAA GCC TTC ACA CTC GC 3'; SEQ ID NO: 20) described in V.a. and WD31 (5' tagaattC GGC cGG AGA CAG CAC GGC GGG TTC GTG TGT GGT TG 3'; SEQ ID NO: 28).). The WD31 primer provides the EcoRI restriction site, the first 5 codons of the sequence encoding the mature α globin chain (an EagI site is created by silent mutation in the fourth codon (CCT modified to CCg)) and the last 6 codons of the sequence of the N-terminal prepropeptide. The PCR amplification and the

treatment of the amplified DNA fragments were carried out as described in the third stage of section II.a. Next, these DNA fragments were doubly digested with HindIII and EagI, purified by electrophoresis on a 1.8% agarose gel and by the action of the "~~Geneclean~~ GENECLEAN™ II" kit (BIO101) and cloned at the HindIII and EagI sites of the dephosphorylated plasmid pBIOC44 described in II.a. The ligation and the transformation were carried out as described in section I.b. The plasmid DNA of the clones obtained, selected on 100 μg/ml ampicillin, was extracted according to the alkaline lysis method (Stephen et al., 1990) and analyzed by enzymatic digestion. The resulting clone was called pBIOC70. The nucleotide sequence of the chimeric gene between the sequence encoding the prepropeptide and the cDNA encoding the mature α globin chain was verified by sequencing as described in section I.b.

Please replace the paragraph beginning at page 48, line 3 with the following new paragraph:

The sequence encoding the N-terminal prepropeptide (PPS) of sporamine A of the tuberized roots of sweet potato was amplified by PCR on the plasmid pMAT103 with the aid of the 2 oligodeoxynucleotides, WD27 (5' gtcattaattaaca ATG AAA GCC TTC ACA CTC GC 3'; SEQ ID NO: 22) described in V.b. and WD32 (5' aatgagct C GGC cGA CTT CTC CTC AGG AGT CAG GTG CAC GGC GGG TTC GTG TGT GGT TG 3'; SEQ ID NO: 29). The WD32 primer provides the SacI restriction site, the first 10 codons of the sequence encoding the mature β globin chain (an EagI restriction site is created by silent mutation in the ninth codon (TCT modified to TCg)) and the last 6 codons of the sequence of the N-terminal prepropeptide. The PCR amplification and the treatment of the amplified DNA fragments were carried out as described in the third stage of section II.a. Next, these DNA fragments were doubly digested with PacI and EagI, purified by electrophoresis on a 1.8% agarose gel and by the action of the "~~Geneclean~~ GENECLEAN™ II" kit (BIO101) and cloned at the PacI and EagI sites of the dephosphorylated plasmid pBIOC45 described in section II.b. The ligation and the transformation were carried out as described in section I.b. The plasmid DNA of the clones obtained, selected on 100 μg/ml ampicillin, was extracted according to the alkaline lysis method

(Stephen et al., 1990) and analyzed by enzymatic digestion. The resulting clone was called pBIOC71. The nucleotide sequence of the chimeric gene between the sequence encoding the prepropeptide and the cDNA encoding the mature β globin chain was verified by sequencing as described in section I.b.